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Research Article

SCREENING OF WOUND HEALING ACTIVITY OF *MADAYANTIKA* (*LAWSONIA INERMIS* LINN.) IN ALBINO WISTAR RATS

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ABSTRACT

Madayantika (*Lawsonia inermis* Linn.) is a miracle medicinal plant used in the treatment of various skin diseases especially in wound healing activity in the Indian system of medicine. **Aim and Objective**: The objective of the study presented in this article was to evaluate the wound healing potential of aqueous extract. The models usually used for evaluation of wound healing activity are Excision wound model, Incision Wound Model and Dead Space model. Among those excisions wound model is selected for the study. Healthy wistar strain Albino rats of either sex weighing 150-200gm were used for the study. Animals are acclimatized in a laboratory and then it were anaesthetized and then dorsal surface of rats were shaved to about an area of 500mm^2 and then it was cut carefully with sterilized forceps and left undressed for open environment, then extracts of test drugs, Scaffolds containing aqueous extract and standard drug were applied and then observed and noted. **Results:** The extract of *Madayantika* (*Lawsonia inermis* Linn.) treated wounds were found to epithelize faster and rate of wound contraction was significantly ($p<0.0001$) increased as compared to control group ($p< 0.01$). The extract treated animals showed from 502 ±0.364 to 25.1±0.252 reduction in wound area when compared with control groups from 502± 0.538 to 40.71± 0.166. **Conclusion:** *Madayantika* (*Lawsonia inermis* Linn.) possess significant wound healing effect which may beneficial in treating wounds. This drug showed extremely significant results in wound healing activity.

KEYWORDS: *Madayantika* (*Lawsonia inermis* Linn.), *Vrana*, Chronic foot ulcers, Excision wound model.

INTRODUCTION

"Pharmacology" is one of the cornerstones of the drug discovery process. "Pharmacology" is the branch of science which deals with study of "Drug action".

"Experimental pharmacology" deals with effect of various pharmacological agents studied on different animal species. Experimental pharmacology involves the study of pharmacology through bioassay to test the efficacy and potency of the drug.

In the case of majority of the drugs it is possible to determine the therapeutic ability based on basic pharmacological and therapeutic uses which were told by our *Acaryas*. From Ancient period onwards only there are number of references available regarding the testing of drugs and foods on the animal for the safety of mankind. In *Caraka Samhita*, *Siddhisthana* (6/79-80) there are numerous references find their way to depict these procedures.[1] *Sushruta Samhita* has dealt with this by allotting a separate chapter in *Sutrasthana* i.e., *Yogyavidhi* (9th chapter), in which it is said that any procedure which is expected to be performed on human being should undergo trials on animals or other things, which has same characteristics and in *Kalpasthana* there is similar discussion dealing with the observation of Animal experiments.[2] *Acarya Vagbhaṭa* has also described them in more or less similar manner. These are not for the drugs and their effects on the animals, but mainly to ascertain possible adverse or poisonous effect.

The drug *Madayantika* (*Lawsonia inermis* Linn.) which is chosen for the study has been mentioned in the context of *Vrana* by *Sushruta.*[3] *Madayantika* (*Lawsonia inermis* Linn.) is having *Kashaya, Tikta rasa, Laghu, Rukshaguna, Shita Vīrya, Kanḍugna* and *Vranaropana* properties which help in formation of healthy granulation tissue, alleviates itching, act as anti- microbial and imparts firmness to skin and flesh.[4]

The main aim of this experimental pharmacology as per ancient scholars and as per modern science is same i.e.

1. To find out the therapeutic agent suitable for human use.
2. Study the toxicity of the drugs.
3. Study the mechanism and site of action of drugs,

Commonly used animals for experimental study are

- Mice
- Rat
- Guinea pig
- Rabbits

Most Commonly used animals are rats because of its

- Availability
- Low cost and
- Small size

Aim of the Study

The present study aims to screen the *In vivo* wound healing activity of scaffolds loaded with *Lawsonia inermis* Linn.

MATERIALS AND METHODS

The aqueous extract of test drug *Madayantika (Lawsonia inermis* Linn.) *Patracurna* is used for experimental purpose and administered to the experimental animals according to the dose required.

Procurement of animals

1. Healthy albino rats of wistar strain of either sex weighing about 150-200 g were used for the study.
2. The animals were acclimatized for a period of one week prior to expose the experimental conditions. Animals were caged individually and kept in an air conditioned room at the temperature of 22±24^{0}C with 50% ± 10% relative humidity with 12 hours light and dark cycle.
3. Throughout the study, rats were maintained at normal laboratory conditions, fed with standard rat pellet diet and drinking water ad libitum.
4. The experiments were carried out after obtaining permission from "Institutional Animal Ethics Committee."

Apparatus

1. Rat feeding needles
2. Weighing Scale
3. Syringes
4. Blunted Forceps
5. Scissors
6. Cotton
7. Gauze

Chemicals

1. Ketamine injection
2. Xylazine injection
3. Betadine ointment (5%)
4. Normal saline
5. Surgical spirit

Route of drug Administration

Route of drug administration plays a pivotal role in Ayurveda. Topical application is one of the routes for administration of drug. This method produces very constant blood levels of the substance, avoiding the need for repeated animal restraint, painful injections, sharp hazards, lower risk of side effects. So, topical application has given utmost importance in the present animal experimentation.

Experimental Model

The model selected to evaluate in vivo wound healing activity are

- Excision wound model[5,6]

Experimental Protocol

The experimental animals were divided into 3 groups containing 6 animals in each group (n=6).

Table No. 4.1. Showing the Experimental Protocol

S.No	Groups	Treatment	Purpose of the study
1.	Disease control	Wound induction by Excision model	To assess the parameters in disease control
2.	Standard	Wound induction by Excision wound model + Povidone Iodine (5%) ointment	To assess the parameters in betadine ointment (5%) treated group
3.	Test	Wound induction by Excision model + *Lawsonia inermis* Linn. scaffolds	To assess the parameters in test treated group.

Excision Wound Model

Healthy wistar strain albino rats of either sex weighing of 150-200gm were used for the study

Animals were housed in a laboratory condition under ambient temperature of 22±24 ^{0} C with 50% ± 10% relative humidity with 12 hrs light and dark cycle. They were fed balanced diet and water ad libtium

Rats were divided into 3 groups containing 6 animals in a group (n=6)

Rats were anaesthetized with Ketamine (80mg/kg I.M) and Xylazine (10mg/kg I.M) prior to experimentation

Dorsal surface of the rats were shaved to avoid interruption of hairs during experimental period

An area of about approximately 500mm^2 was marked on the dorsal surface of the rat and then the marked skin was cut carefully with help of sterilized forceps

Wounds were left undressed to open environment

Then the animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced with other animals

The extracts of test drugs, scaffolds containing aqueous extract of *Lawsonia inermis* Linn., the standard drug betadine (5%) ointment were applied on the wound area immediately

Wound dressing was done once a day after washing the wound carefully with 0.9% saline water and mopping with the sterile non-absorbable cotton till the wound was completely healed

The scaffolds containing *Lawsonia inermis* Linn., betadine ointment (5%) were applied on the wound area and the animals were observed for wound closure at 0day, 7thdayand 15thday and for period of epithelialization

The progressive changes in wound area were monitored by a camera for every 5 days

Statistical Analysis

All the data was expressed as mean ± SEM. Statistical significance between more than 2 groups was tested using one way ANOVA followed by the Dennett's test. Statistical significance was determined at p <0.05.

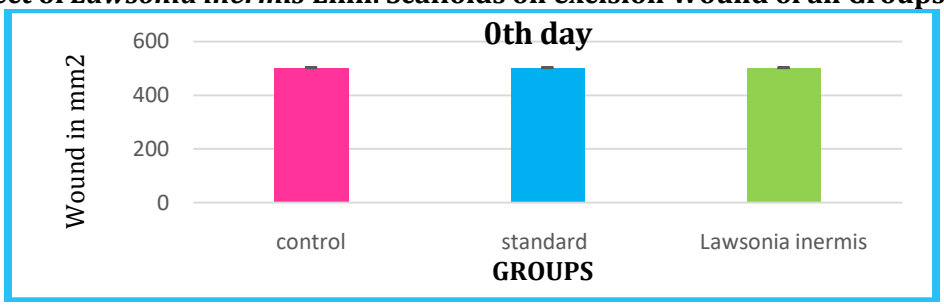
RESULTS

Table 1: Effect of *Lawsonia inermis* Linn. Scaffolds on excision Wound of all Groups

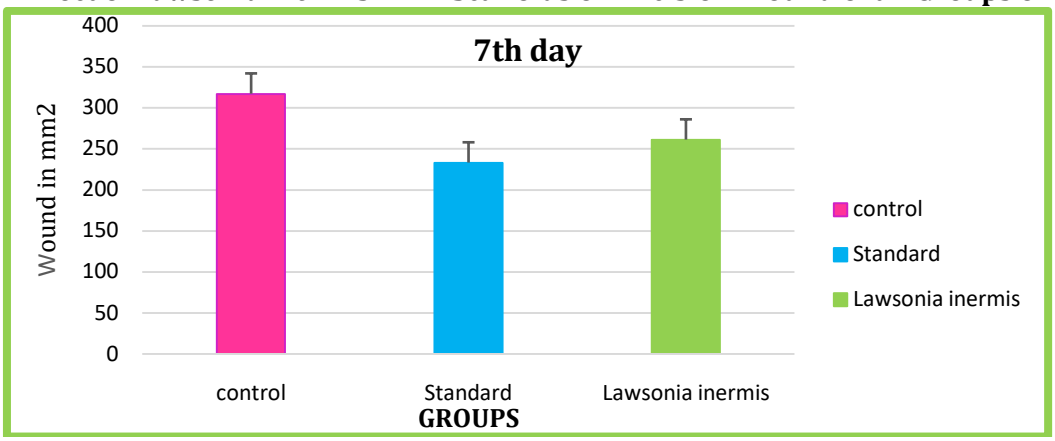
S.No.	Groups	Wound area in mm^2		
		0 day	7 th day	15 th day
1.	Control	502± 0.538	317 ±1.72	40.71± 0.166
2.	Standard	502±0.367 ^{ns}	233±0.728***	15.3± 0.098***
3.	*Lawsonia inermis* Linn.	502 ±0.364 ^{ns}	261±0.372**	25.1±0.252***

***p<0.0001,**p<0.001, ns when compared with control group

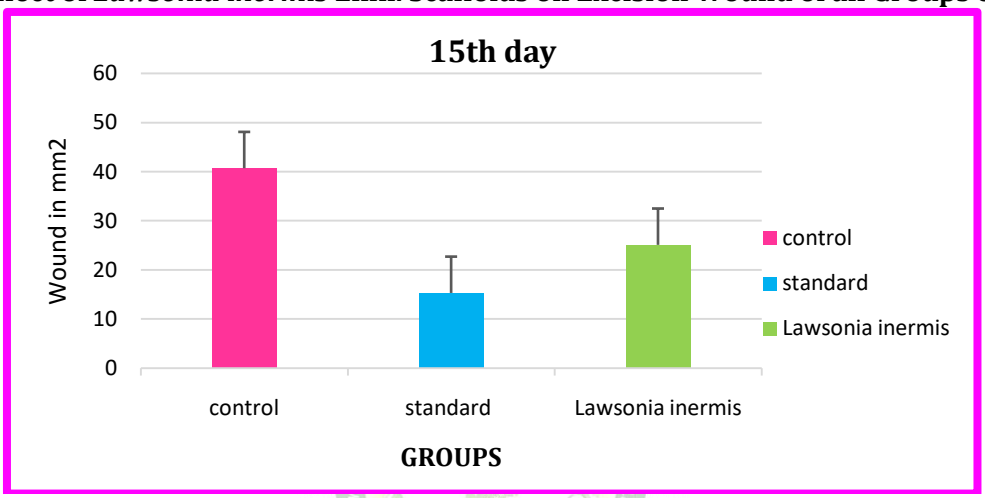
Graph 1: Effect of *Lawsonia inermis* Linn. Scaffolds on excision Wound of all Groups on Zero day



Graph 2: Effect of *Lawsonia inermis* Linn. Scaffolds on Excision Wound of all Groups on 7th day



Graph 3: Effect of *Lawsonia inermis* Linn. Scaffolds on Excision Wound of all Groups on 15th day



Excision wound Model Images

Zero days

Fig 1: Disease control

Fig 2: Betadine (5%)

Fig 3: *Lawsonia inermis* Linn. treated

7th day

Fig 4: Disease control

Fig 5: Betadine ointment (5%)

Fig 6: *Lawsonia inermis* Linn. Treated

15th day

Fig 7: Disease control

Fig 8: Betadine ointment (5%)

Fig 9: *Lawsonia inermis* Linn. treated

Pictures of *Madayantika* (*Lawsonia inermis* Linn.)

Fig 10: *Madayantika* plant

Fig 11: *Madayantika* Leaf

Fig 12: *Madayantika* Flowers

Fig 13: *Madayantika* Fruits

DISCUSSION

Wounds are physical injuries that result in an opening or breaching of the skin leads to disruption of normal physiological state of skin. In order to maintain normal homeostasis proper healing of wound is essential for the restoration of disrupted anatomical stability and functional status of the skin. Wound healing is an intricate process in which the skin itself repairs the injury in sequential events which involves inflammation, proliferation and remodeling.

Whenever there is a breach to the skin integrity occurs, platelets are the first cells responds to the injury and initiate wound healing through fibrin clot formation. In mean while inflammatory process begins with recruitment of inflammatory mediators such as neutrophils, macrophages etc., to the injured site. Neutrophils acts as chemo attractants for other cells involved in inflammation and it also releases mediators such as TNF-α and interleukins.[7] Macrophages play a multiple roles by releasing cytokines which promotes inflammatory response by recruiting other additional leucocytes.[8]

In proliferative phase, the main wound healing process lies in cells like fibroblasts and endothelial cells play an important role in collagen synthesis, formation of blood vessels and formation

of granulation tissue at the injury site. Tissue repair process is mediated by the release of growth factors (EGF, PDGF, VEGF). The released growth factors promote cell proliferation angiogenesis and synthesis ECM molecules by resident skin cells.[9]

Remodeling phase is the last phase of wound healing process occurs from 21st day to upto 1 year after injury. The formation of granulation tissue stops and leads to wound contraction by them fibroblasts by attaching to collagen.

Present study is mainly focused to screen *invivo* wound healing activity of scaffold loaded with *Madayantika* (*Lawsonia inermis* Linn.), It was observed that scaffold loaded with *Lawsonia inermis* Linn. treated groups showed significant increase in wound closure time by enhancing epithelization when compared with that of control group and wound repair in *Lawsonia inermis* Linn. treated group started from the 5th day onwards. This prominent increase in epithelisation may be due to the mitogenic property of the *Madayantika* (*Lawsonia inermis* Linn). treated groups and also the presence of various phytochemical constituents like Tannins, Flavanoids, Terpenoids and Saponins, which have anti-bactrerial, anti-inflammatory, anti-fungal activity. Metal analysis showed the presence of sodium, potassium, iron, manganese, chromium in leaf powder of *Madayantika (Lawsonia inermis* Linn*)*. Sodium helps in electrolyte balance in the body where as potassium is helpful in muscle contraction such that it drains out the fluid and helps in muscle contraction such that it drains out the fluid and helps in formation of healthy granulation tissue. Copper enable the body to form RBC and blood vessels and also plays key role in angiogenesis. Iron is having potent anti-oxidant wound healing. Chromium plays key role in angiogenesis. Manganese activates a enzyme prolidase which has the collagen formation effect so it helps in wound healing. In this way sodium, potassium, iron, copper, chromium and manganese in *Madayantika* (*Lawsonia inermis* Linn). plays significant role in wound healing. Since, these ion's are important for wound healing.

CONCLUSION

The wound healing property of leaf extract of *Madayantika* (*Lawsonia inermis* Linn.) appears to be due to the presence of its active principles which accelerates the healing process. The present study has demonstrated that an aqueous extract of *Madayantika* (*Lawsonia inermis* Linn.), leaves has properties that render it capable of promoting wound healing. Finally, it may be concluded that *Madayantika* (*Lawsonia inermis* Linn). Possess significant wound healing effect which may beneficial in treating wounds. This drug can be further investigated for other types of wounds and it may prove boon to many chronic wounds.

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